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## Treg cells in cancer: Where are we now?

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## **Abstract**

There have been substantial strides forward in our understanding of the contribution of regulatory T ( $T_{\text{reg}}$ ) cells to cancer immunosuppression. In this issue we present a series of papers highlighting the emerging themes on this topic relevant not only to our understanding of the fundamental biology of tumour immunosuppression but to the design of new immunotherapeutic approaches. The substantially shared biology of  $CD4^+$  conventional T ( $T_{\text{conv}}$ ) and  $T_{\text{reg}}$  cells necessitates a detailed understanding of the often opposing functional consequences that immunotherapies will have on  $T_{\text{reg}}$  and  $T_{\text{conv}}$  cells, a prominent example being the potential for  $T_{\text{reg}}$ -mediated hyperprogressive disease following anti-PD-1 therapy. Such understanding will aid patient stratification and the rational design of combination therapies. It is now becoming clear that  $T_{\text{reg}}$  cells within tumours also exhibit distinct biological features to both  $T_{\text{conv}}$  cells and to  $T_{\text{reg}}$  cells in other tissues. These distinct features provide the opportunity for development of targeted immunotherapies with greater efficacy and reduced potential for inducing systemic toxicity.

## Main text

T cells have an ability to recognise and kill cancer cells but their function is often suppressed within tumours. Whereas CD4<sup>+</sup> and CD8<sup>+</sup> conventional T (T<sub>conv</sub>) cells promote immune activation, CD4<sup>+</sup> regulatory T (T<sub>reg</sub>) cells, dependent upon the transcription factor Foxp3, suppress T<sub>conv</sub> cell responses and are required for immune homeostasis in both mice and humans<sup>1, 2</sup>. It is now clear however that beyond this beneficial function, T<sub>reg</sub> cells can cause profound suppression of immune function within tumours<sup>3, 4</sup>. In a variety of murine tumour models, ablation of T<sub>reg</sub> cells results in activation of CD4<sup>+</sup> or CD8<sup>+</sup> T<sub>conv</sub> cells and rejection of solid tumours<sup>5, 6, 7, 8</sup>. Moreover, high T<sub>reg</sub> ratios relative to total T cells or CD8<sup>+</sup> T<sub>conv</sub> cells are associated with poorer survival in breast cancer<sup>9</sup>, non-small cell lung carcinoma<sup>10</sup>, hepatocellular carcinoma<sup>11</sup>, ovarian cancer<sup>12, 13</sup>, renal cell carcinoma<sup>14</sup>, pancreatic cancer<sup>15</sup>, colorectal carcinoma<sup>16</sup>, gastric cancer<sup>17</sup> and cervical cancer<sup>18</sup>. An understanding of a powerful role of T<sub>reg</sub> cells in tumour immunosuppression is thus emerging with extensive evidence from experimental mouse models complemented by a growing body of evidence in human cancer. In this Review series, we consider the progress made in our understanding of the mechanisms that lead to the accumulation and suppressive function of T<sub>reg</sub> cells within tumours, the unique properties of tumour-infiltrating T<sub>reg</sub> cells, and our means to selectively target them in cancer.

While their immunosuppressive function make T<sub>reg</sub> cells are an attractive target for specifically directed therapy, it is also important to consider the effects upon T<sub>reg</sub> cells of conventional immunotherapies thought to primarily target T<sub>conv</sub> cells. Despite striking efficacy in some cases, therapies targeting PD-1/PD-L1 signaling are ineffective at inducing durable responses in a majority

of patients, and can induce rapidly progressive disease referred to as ‘hyperprogression’ in a minority of patients<sup>19, 20</sup>. A recent study suggests that hyperprogression is in part attributable to blockade of PD-1 signaling on Treg cells which, in susceptible individuals, results in enhanced Treg suppressive function<sup>21</sup>. It remains to be determined whether a similar phenomenon underlies poor clinical responses to PD-1 therapy in subsets of patients but the findings highlight the need to consider the opposing effects that immunotherapies may have on the Tconv and Treg compartments. Indeed, such consideration may provide a basis for patient stratification or the rational design of combination immunotherapy. Evidence from both mouse models<sup>22</sup> and human cancer patients<sup>23</sup> indicate that the activity of anti-CTLA-4 therapy is in part attributable to antibody-dependent cellular cytotoxicity (ADCC)-mediated depletion of intratumoural Treg cells, which express high levels of CTLA-4. Indeed, in patients with advanced melanoma, favourable response to treatment with the anti-CTLA4 monoclonal antibody ipilimumab was associated, among patients with inflamed tumours, with the presence of a coding polymorphism within CD16a/FcγRIIIa which results in its higher affinity for Fc suggesting that FcγRIIIa-dependent ADCC activity is involved in the efficacy of ipilimumab therapy in humans<sup>23</sup>. As **Lim et al.** point out, Treg and Teff cells have substantially shared intracellular signaling pathways and the balance to which distinct immunomodulatory agents affect Treg suppression *versus* Teff cell-mediated anti-tumour immunity determines their net effect upon tumour progression as is exemplified by the net immunostimulatory effect of genetic or pharmacological disruption of PI3Kδ activity<sup>24</sup>. Indeed, it is likely that shared expression of CD25 and CCR4 on tumour-infiltrating Treg cells and activated Tconv cells have contributed to the lack of robust clinical efficacy of antibody reagents targeting these molecules.

Finally, as discussed by Yano *et al.*, checkpoint immunotherapy may result in reactive recruitment of Treg cells to tumours in response to increased inflammation<sup>25</sup>. Thus, a theme emerging from a number of reviews in this series is the substantially shared biology of T<sub>reg</sub> and T<sub>conv</sub> cells and the need to consider the effects of therapy on both the T<sub>reg</sub> and T<sub>conv</sub> compartments.

Treg cell frequency in the tumour immune infiltrate often far exceeds that in normal tissues, suggesting that co-option of T<sub>reg</sub> cells by tumours is an important feature of cancer development and a requisite for cancer progression in number of tumour types. Stockis *et al.* consider the mechanisms that drive T<sub>reg</sub> accumulation within tumours, reviewing our understanding of the molecular basis for recruitment and maintenance of Treg cells within tumours, and proposing that selective recruitment of thymic Treg (tT<sub>reg</sub>) cells rather than *de novo* induction of induced T<sub>reg</sub> (iT<sub>reg</sub>) cells is the dominant mechanism by which Treg cells accumulate in cancer<sup>26</sup>. While experimental observations supportive of this conclusion are presented, the relative functional contribution of tT<sub>reg</sub> and iT<sub>reg</sub> cells to tumour immunosuppression has yet to be formally established. T<sub>reg</sub> cells within tumours express high levels of specific chemokine receptors, such as CCR2, CCR4, CCR8 and CCR10, and it is plausible, though again not clearly established, that expression of these receptors drive recruitment of Treg cells into tumours. In addition, the association of tumours with tertiary lymphoid structures contributes to recruitment of Treg cells into tumours<sup>27</sup>. The tumour environment provides an environment supportive of Treg cell proliferation and Stockis *et al.*, also review the role of co-stimulatory and co-inhibitory receptor and cytokine signaling on T<sub>reg</sub> cell maintenance and activation in tumours. It is clear, however, that much more work is needed to better dissect the distinct functions

of chemokine, cytokine and co-stimulatory/co-inhibitory receptors on T<sub>reg</sub> and T<sub>conv</sub> cell migration and function, respectively.

Given the shared involvement of T<sub>reg</sub> cells in immunological tolerance and tumour immunosuppression, selective targeting of T<sub>reg</sub> cells in tumours is desirable but requires an understanding of their specific biological characteristics. Yano *et al.* consider the specific molecular and functional characteristics of T<sub>reg</sub> cells in tumours, observing that a number of molecular, cellular and metabolic characteristics distinguish them from T<sub>reg</sub> cells in other tissues<sup>25</sup>. Morena Ayala *et al.* describe attempts made to target the immunosuppressive function of T<sub>reg</sub> cells in preclinical mouse tumour models and in the clinic<sup>27</sup>. Such attempts include systemic administration of P300/HAT, EZH2 and BET inhibitors whose consequence upon Treg cell suppression results in augmented tumour immunity. Zaiss *et al.* review the role of tissue-resident Treg cells in promoting both non-immune processes and immune processes associated with wound healing. In part, this activity is mediated by release of the EGF-like growth factor Amphiregulin by Treg cells whose activity extends beyond its canonical function in wound repair to promoting the release of bioactive TGF- $\beta$  through inside-out activation of integrins. The extent to which the function of Treg-derived Areg in promoting tumour immunosuppression involves canonical and non-canonical Areg functions have yet to be determined<sup>28</sup>.

In summary, there have been substantial strides in our understanding of the contribution of T<sub>reg</sub> cells to tumour immunosuppression. A detailed understanding of the often opposing effects of

immunotherapies on both the  $T_{\text{conv}}$  and  $T_{\text{reg}}$  compartments will aid the design of new immunotherapy approaches and the interpretation of their outcomes. In addition, there is a growing awareness of the involvement of  $T_{\text{reg}}$  cells in influencing the outcome conventional checkpoint inhibitor therapy responses, with potential functional contributions as profound and deleterious as anti-PD-1-induced hyperprogression. In this context, the shared biology of Treg and Tconv cells presents both an obstacle and an opportunity, especially for patient stratification and rational design of combination immunotherapies. The observation that  $T_{\text{reg}}$  cells in tumours harbour distinct molecular profiles that contribute to their selective migration and function and that distinguish them from  $T_{\text{reg}}$  cells in other tissues provide extremely important opportunities for the selective targeting of Treg cells in cancer.

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